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TEST OF THE FEASIBILITY AND EFFECTS OF LONG-TERM INTRAMUSCULAR IMPLANTATION OF ARCHIVAL TAGS IN PELAGIC FISHES USING SCALE MODEL TAGS AND CAPTIVE JUVENILE YELLOWFIN TUNA (*THUNNUS ALBACARES*)

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ABSTRACT

Electronic archival tags capable of measuring and storing (for up to 12 years) data on ambient light level, a fish's depth, and water temperature (from which geographic positions can be estimated) are now commercially available. Although many of the engineering problems have been surmounted, long-term (months to years) tag attachment methods remain problematic for tunas (*Thunnus* spp.) and especially billfishes (*Istiophoridae* and *Xiphiidae*) where large individuals (> 200 kg body mass) can be difficult to restrain or safely remove from the water. Intramuscular placement of archival tags in these fishes could be a desirable method of attachment. Therefore, we tested the feasibility of long-term intramuscular implantation of archival tags in large pelagic fishes by placing stainless steel scale model tags into juvenile yellowfin tuna (*Thunnus albacares*) held in shoreside tanks. We found model archival tags can be easily implanted within the muscle, remain near the body surface as the fish grow, and can be carried for 10 months without causing infection or adverse tissue reactions.

INTRODUCTION

Understanding the movements and effects of oceanographic conditions on the distribution and fishing gear vulnerability of large commercially valuable tunas (*Thunnus* spp.) and billfishes (*Istiophoridae* and *Xiphiidae*) is important to population assessments, the prediction of fisheries interactions, international fisheries management, and resource conservation (Joseph & Greenough, 1979; Hanamoto, 1987; Brill, 1994; Hinton & Nakano, 1996). One of the most promising techniques for monitoring long-term movements and depth distributions of pelagic fishes is the use of electronic archival tags (Klimley et al., 1994; Boehlert, 1997). Archival tags can be set to record swimming depth, water temperature, and ambient light level (i.e., times of sunrise and sunset) from which geographic position of the fish can be estimated. To date, the engineering problems have been surmounted and archival tags are now commercially available.

Gunn et al. (1994) and Block et al. (1997) have successfully implanted archival tags in the peritoneal cavity of Southern and Northern bluefin tuna (*Thunnus maccoyii*, Castelnau and *T. thynnus*, L., respectively), and Tyus (1988) has demonstrated that radio tags can be retained for years when inserted into the peritoneal cavity of two species of freshwater fishes. Other studies (Summerfelt & Mosier, 1984; Chisholm & Hubert, 1985; Vogelbein & Overstreet, 1987; Marty & Summerfelt, 1986; 1988; Lucas, 1989; Knights & Lasee, 1996; Kynard & Kieffer, 1997), however, have shown that ultrasonic and radio transmitters placed into the peritoneal cavity of fishes can be lost through trans-intestinal or body wall expulsion, and can cause a significant inflammatory response and adverse morphological effects. More important, intraperitoneal placement of archival tags may not be practical in all circumstances; large (> 200 kg body mass) tunas and especially billfishes can be difficult to restrain or remove from the water without risk of significant injury to the fish or to the crew attempting to implant the tag.

An alternative approach is intramuscular implantation. In this manner, archival tags could be implanted with minimal restraint and without removing the fish from the water. Roberts et al. (1973a; 1973b; 1973c) and Vogelbein & Overstreet (1987) have shown that tissue reactions to long-term implantation of plastic devices in salmon (*Salmo salar*, L.) and two species of estuarine teleosts (spot, *Leiostomus xanthurus*, Lacepède and seatrout, *Cynoscion nebulosus*, Cuvier) can be severe and include necrosis and extensive fibrogranulation. Although adverse tissue reaction is clearly a critical issue for the successful intramuscular deployment of archival tags in fish such as tunas and billfishes, it has not been investigated in these species. Therefore, we examined the tissue reactions of captive juvenile yellowfin tuna (*Thunnus albacares*, Bonnaterre) to intramuscular implantation of scale model archival tags to test the feasibility of long-term intramuscular implantation of archival tags in large pelagic fishes.

Our primary objective was very specific, to determine the tissue reactions to long-term intramuscular implantation of archival tags and thus begin to ascertain the feasibility of intramuscular implantation of archival tags in large pelagic fishes. Because of the difficulties

and expenses associated with obtaining and maintaining live yellowfin tuna for extended periods, we were limited to working with relatively few fish. Moreover, we could not realistically mimic intramuscular implantation procedures likely to be used at sea because these are yet to be developed. From the outset, therefore, we felt it obviously impractical to design a study to determine the effects of intramuscular tag implantation on mortality or growth rates, or the effects of specific insertion methods on tag retention.

MATERIALS AND METHODS

All current commercially available functional archival tags are too large to be implanted in the 1-2 kg yellowfin tuna (*Thunnus albacares*) normally maintained in shoreside tanks at the Kewalo Research Facility (National Marine Fisheries Service Honolulu Laboratory, Southwest Fisheries Science Center, NOAA) and available for our study. We, therefore, employed $\approx 1/25^{th}$ scale model tags supplied by Northwest Marine Technology Inc. (P.O. Box 427, Ben Nevis Rd., Shaw Island, Washington 98286, USA), a manufacturer of archival tags. Model archival tags were approximately 0.6 cm in diameter, 4 cm long, weighed 3.5 g in air, and were made of the same 316 grade stainless steel as the larger functional archival tags. The model tags also had a simulated fiber-optic light stalk, crafted from nylon monofilament line encased in Teflon, on one end. In a functional archival tag, the flexible fiber-optic light stalk must extend out through the skin to monitor ambient light levels.

Yellowfin tuna, weighing between 1 and 2 kg, were purchased from local commercial fishermen and initially held for several weeks in 8-m diameter above-ground pools. During this period, animals were trained to accept dead food and allowed to recover from any injuries incurred during capture and the transfer to shoreside tanks. To minimize stress and the risk of skin or fin damage, no attempts were made to weigh or measure the fish, or to apply separate identification tags. After model archival tags were implanted, fish were transferred and held for approximately 10 months in a 13 m by 23 m by 2 m (deep) oceanarium. All tanks were supplied with aerated sea water. Water temperature was not controlled and varied between 24-26°C. The fish were fed chopped squid daily. Tuna holding procedures at the Kewalo Research Facility are more fully described by Brill (1982).

Fifteen fish were tagged. In 13 fish, the model archival tags were inserted either perpendicular or roughly parallel to the dorsal body surface, but with the simulated fiber optic light stalk pointed toward the fish's head. In the remaining two fish, tags were inserted roughly parallel to the dorsal body surface but with simulated fiber optic light stalk pointed toward the tail. It was not possible, however, to determine the exact pathway (i.e., orientation relative to the dorsal body surface) the tag body followed when inserted into the musculature. To apply the tags, fish were individually dip-netted then lightly restrained on a foam rubber padded operating table covered with a wet plastic sheet. A small incision (no more than 0.5 cm) was made using a scalpel at a point approximately midway between the anterior margins of the first and second dorsal fins. The incision was through the skin only and did not penetrate into the musculature. The tags were then manually inserted through this opening. They slid easily into the muscle with very little force. Entry wounds were quickly closed with commercial tissue adhesive (Vet Bond,

3M Animal Care Products, St. Paul, Minnesota), as recommended by Nemetz & MacMillan (1988). The entire procedure, from netting to release back to the holding tank, generally took less than 1 minute. Fish showed no obvious acute responses to the tagging procedure; they swam and schooled normally immediately after release.

At the conclusion of the experiment, fish were all sacrificed on the same day, weighed and fork lengths measured. The external tag sites were photographed, tissues surrounding the tag site dissected and photographed, and samples taken. Tissue samples were cut into 1 centimeter cubes and fixed in a 0.2 M sodium cacodylate-buffered 10% formalin solution. After 14 hours of primary fixation at 4°C, samples were rinsed three times in 0.2 M sodium cacodylate buffer and dehydrated in a series of 10-minute ethanol rinses (30%, 50% 70%, 85%, 95%, and twice in absolute ethanol). Samples were subsequently subjected to a series of graded changes from absolute ethanol to absolute xylene and embedded in paraffin wax with graded exchanges of xylene with heated paraffin. Semi-thin sections (6 μm) were cut with a microtome, dewaxed, and hydrated prior to staining with hematoxylin. After staining, the sections were dehydrated, mounted using Protexx (Baxter Diagnostics Inc., Scientific Products Division, McGraw Park, Illinois), and photographed using a Zeiss IIIRS light microscope.

RESULTS

Ten of the 15 fish survived until the sampling date. Prior to sampling, all fish were voraciously feeding and appeared in good health. Two fish died within days of tagging and these 2 were the fish that had their tags inserted with the simulated fiber optic light stalk pointed toward the tail. The tags had become dislodged and were in the process of being shed when the fish succumbed. Two fish died approximately 5 months after tagging, and the third and final mortality occurred approximately 9 months after tagging. No specific cause could be assigned to the three latter mortalities. Growth was substantial in all surviving fish (Fig. 1a). Whereas fish weighed approximately 1-2 kg when the tags were inserted, mean (± SD) and range of body mass at the conclusion of the experiment were 11 ± 2 and 6-15 kg, respectively.

Of the 10 fish that survived until the conclusion of the experiment, 7 retained their tags. In the fish that retained their tags, there was no macroscopic evidence of infection or tissue necrosis around the tag insertion site or where the simulated fiber-optic light stalk exited the skin (Fig. 1b). Tag bodies were all found to be embedded approximately 5 cm below the dorsal body surface. In 6 fish, the tag bodies were roughly parallel or at an acute angle to the dorsal body surface (Fig. 1c). The portion of the simulated fiber-optic light stalk that was embedded within the muscle pointed toward the head while the exposed portion bent back toward the tail. In the seventh fish, the tag body was perpendicular to the dorsal body surface and there was a healed scar in the epidermal layer inferior from the tag stalk. There was no evidence that any of the tagged fish would lose their tags in the near future. In the 3 fish that shed their tags, initial tag sites could not be positively identified. Because it is not possible to catch, restrain, and examine the fish while in the large oceanarium without extreme stress to the animal, we could not determine when tags were actually shed.

In all fish, the majority of the muscle fibers surrounding the tag body and simulated fiber-optic light stalk appeared normal. There were, however, some minor signs of bleeding or necrotic muscle immediately adjacent to both (Fig. 2a, f). The bleeding may have actually occurred postmortem during dissection, however. There was no evidence of metal corrosion in any sample, as has been reported to occur with stainless steel implants in mammals (Williams and Roaf, 1973).

The only prominent tissue reaction revealed by histological examination was the development of layers of connective tissue surrounding the embedded objects (Fig. 2). These most likely functioned to protect the surrounding muscle and were composed of both loose and dense connective tissues. The loose connective tissue was heavily vascularized (Fig. 2f) and typically consisted of numerous fibroblasts as well as reticular fibers (Fig. 2d, f). The dense connective tissue, immediately adjacent to the embedded objects, was predominantly composed of parallel fibers and usually consisted of 6 to 20 cell layers, but its thickness varied between fish. It was often thicker on one side of the embedded objects than on the other (Fig. 2a, b, c).

DISCUSSION

We made no attempt to ensure sterility of the tags so as to mimic situations likely to occur during shipboard tagging operations. However, it is unlikely that intramuscular tag implantation at sea will employ a simple skin incision, manual insertion of tags, and wound closure with tissue adhesive. Most likely a yet-to-be-developed mechanical device will be required. It is our subjective impression, however, that optimal procedures would involve inserting archival tags an acute angle to the dorsal body surface with the fiber optic light stalk pointing anteriorly.

Over a 10-month period, the yellowfin tuna increased in body mass by up to approximately an order of magnitude, yet the tag still retained almost its original position relative to the body surface, and nearly the full length of the simulated fiber-optic stalk remained exposed. This is significant for two reasons. First, if the fish's muscle grew completely around the functional fiber-optic light stalk, an archival tag could no longer record light levels (i.e., times of sunrise and sunset), and geographic position could not be estimated from the recorded data. Second, without some external indication of the presence of a tag, either from a protruding fiber optic light stalk or a separate identification tag, returns of the archival tags from recaptured fish would be much less likely.

In the fish that retained the model archival tags, we found no evidence of serious infection, chronic inflammation, or tissue necrosis adjacent to the tag site. Instead, only a thin protective layer of connective tissue surrounded both the tag body and the portion of the simulated fiber-optic light stalk within the muscle (Fig. 2). Admittedly, this lack of serious tissue response could be due to presumably lower activity levels of yellowfin tuna in captivity versus in the open ocean. Activity levels have been shown to affect the severity of induced tagging lesions in the muscle of salmon (Marty & Roberts, 1976). Routine swimming speeds of juvenile yellowfin tuna in captivity and observed by acoustic telemetry in the open ocean are, however, not drastically different (Magnuson, 1978; Holland et al., 1990). Moreover, tunas held in the

large oceanarium at the Kewalo Research Facility increase their activity during feeding frenzies enough to increase their deep muscle temperatures by up to 6°C (from a normal 2-4°C above ambient to up to 8°C above ambient, Brill et al., 1994).

The fibrous cell layers we observed surrounding the tag body and simulated fiber-optic light stalk are comparable to those seen in similar studies on fish (Roberts et al., 1973a; 1973b). The differentiation of mesenchymal cells into fibrous connective tissue occurs in response to a prolonged stress, such as the insertion of a foreign object, and it is not a phenomenon unique to fish muscle, however. An increase in fibrous tissue, with or without an accompanying inflammatory cellular response, has been described in mammalian tissues and is commonly referred to as the 'metal reaction.' In mammals, the density of fibrous tissue developed often varies between fish and upon the type of stress (Williams & Roaf, 1973).

Our findings of only minor necrosis in yellowfin tuna muscle immediately adjacent to the stainless steel tag body and simulated fiber-optic light stalk differs from studies where investigators used nylon filament tags. The long-term intramuscular implantation of nylon filament tags in salmon and seatrout causes significant widespread lesions in the epidermis, dermis, and muscle resulting in serious bacterial and fungal infections (Roberts et al., 1973a; 1973b; 1973c; Vogelbein & Overstreet, 1987). Furthermore, in some fish species significant muscle cell degeneration and replacement with fibrous tissue (i.e., fibrogranulation) occurs (Finn & Nielson, 1971; Mawdesley-Thomas & Bucke, 1972; Roberts et al., 1973a), something we did not observe with the model stainless steel archival tags. In our study, the lack of cellular damage surrounding each tag site indicated that the model archival tags remained in a constant position within the muscle. If the tags moved within the muscle, they would have most likely caused a more noticeable response. The observation that the tag body remained relatively close to the body surface also supports the contention that the tag body did not migrate.

In summary, our results demonstrate that intramuscular implantation of archival tags could be a viable alternative to intraperitoneal implantation in larger fishes, although neither intraperitoneal nor intramuscular implantation is likely to be completely free of adverse consequences. Moreover, currently available functional archival tags are significantly larger than the scale models used in our study. For example, functional Northwest Marine Technology archival tags are cylindrical (16 mm in diameter and 100 mm long) and weigh 56 grams in air, although smaller models are currently under development. Whether functional archival tags implanted intramuscularly in large tunas, marlin, and swordfish will be as benign as the small scale model tags we implanted in juvenile yellowfin tuna remains to be learned. However, with a tag return rate in pelagic fishes of usually only a few percent (Bayliff and Holland, 1986; Hunter et al., 1986), an “at sea” study of the effects of intramuscular implantation of full-size model archival tags using recaptured fishes will be difficult.

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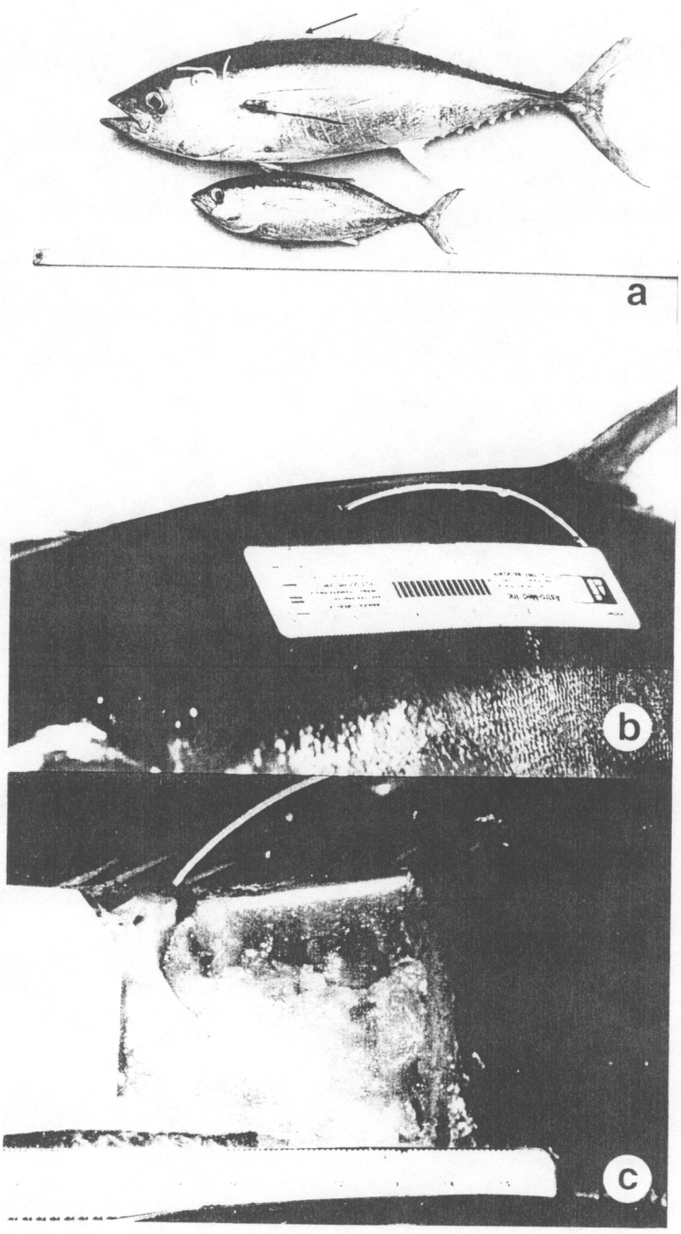

Figure 1.--(a) A difference in body size is seen between a newly captured yellowfin tuna and one that had been in captivity at the Kewalo Research Facility for 10 months. The smaller animal weighs approximately 1-2 kg and is representative of the size of the fish at the beginning of the study. The larger animal is representative of a captive turna that had a model archival tag embedded in its dorsal musculature. A portion of the simulated fiber-optic stalk is seen protruding from the dorsal body surface (arrow). (b) Photograph showing the exit site of the simulated fiber-optic stalk through the dorsal body surface. No evidence of infection or external damage near the stalk was noted in any fish. (c) A gross dissection exposing the placement of the model archival tag and simulated fiber-optic light stalk in the dorsal musculature. In all fish retaining their tags, the tags were firmly embedded in the white muscle and there was no macroscopic evidence of infection, excessive scarring, or hemorrhage.

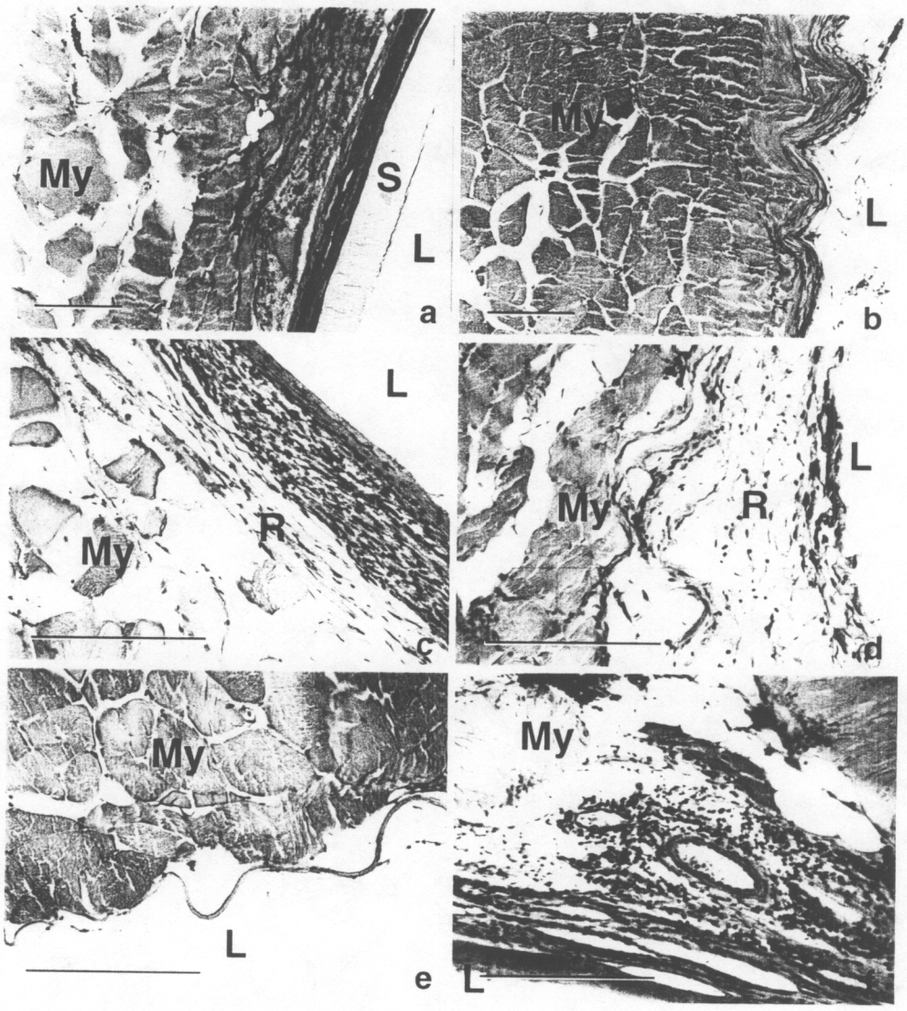

Figure 2.--Light micrographs of semi-thin sections (6 μm) through epidermal and muscle tissues surrounding simulated fiber-optic light stalk and model archival tag in yellowfin tuna (Scale = 100 μm). (a) A protective layer of cells separates the myofibrils (My) from the simulated fiber-optic light stalk (S). This protective cell layer is composed of both loose and dense connective tissues (L = lumen of the simulated fiber-optic light stalk; original magnification 63X). (b) A similar layer of cells is noted between the tag body lesion (L) and the healthy muscle (My) (original magnification 63X). (c, d, and e) The layers of dense and loose connective tissue vary in thickness between fish. The loose connective tissue typically consists of numerous fibroblasts and reticular fibers (R) (L = tag body lesion) original magnification 100X). (f) Vessels with red blood cells are commonly seen in the loose connective tissue (original magnification 100X).